

sub C14
A. Z. Zund
4
3
Claim 7 (amended) The process of Claim 6, wherein said [shellfish is] oysters are
enclosed in liquid-permeable bags filled with pressurizable liquid prior to exposing said
[shellfish] oysters to hydrostatic pressure.

Please cancel Claims 8-26 without prejudice.

REMARKS

This is a response to the Office Action of July 21, 1999. Claims 1-26 are pending in the application. Claims 5, 8-26 were cancelled by the applicant. The applicant reserves the right to file divisional applications(s) on the non-elected claims prior to termination of proceedings in the instant application.

Claims 1-4 and 6-7 were rejected under 35 USC 112, first paragraph. The Examiner contends that the specification does not provide sufficient disclosure for elimination of bacteria in raw shellfish. Further, the Examiner contends that the specification discloses pressure values suitable for shucking of oysters but not for elimination of bacteria in oysters. Finally, the Examiner cited U.S. Patent No. 5,593,714 for a proposition that pressures treatments of 25,000 p.s.i. do not eliminate pathogenic bacteria in shellfish.

The applicant respectfully traverses this rejection and submits that throughout specification, the applicant stressed that the method in accordance with the instant invention is beneficial for two purposes: elimination of pathogenic naturally-occurring bacteria, such as *Vibrio Vulnificus*, and shucking of oysters. It was the former purpose that was the original goal of the applicant. The shucking of oysters was a phenomenon that was not anticipated or predicted at the start of numerous experiments and tests

conducted by the applicant. The applicant respectfully draws attention of the Examiner to for example, page 6 of the specification, wherein the subject of bacteria elimination is discussed alongside with the discussion of oyster shucking.

The applicant submits herewith a letter from Dr. Marilyn B. Kilgen, Head of the Department of Biological Sciences of Nicholls State University. Dr. Killgen works totally independent of the applicant; she is employed by the University. Dr. Kilgen's letter discusses in detail the need for elimination of pathogenic bacteria from raw shellfish, such as oysters.

As Dr. Kilgen stated, there is a significant difference between the processes of "pasteurization" and "sterilization." Persons skilled in the art of biological sciences, as Dr. Kilgen undoubtedly is, understand that sterilization kills all living organisms and microorganisms in a product, regardless of whether the organism is pathogenic (capable of causing disease) or not.

Patent No. 5,593,714 is directed to "sterilization" of food products. Please note Abstract, line 1; column 1, lines 5 – 9; column 2, lines 44 – 50; column 3, lines 1-2, column 6, lines 53 – 55, etc. The '714 patent is not directed to elimination of only pathogenic organisms; on the contrary, the teaching of the patent is to eliminate all micro-organisms, macro-organisms, spores, viruses, etc. Therefore, the teachings of the '714 patent cannot be fully applied to the instant invention.

Dr. Kilgen's letter also describes the tests that she and her team performed at the request of the applicant to test efficacy of his method. It is merely coincidental that Dr. Kilgen used oysters and not other molluscan shellfish, such as clams and others.

As Dr. Kilgen states, *Vibrio Vulnificus*, the pathogenic organism designed to be eliminated with the process of the instant invention, is a naturally-occurring marine bacteria. This organism caused a significant concern of the public as being the cause of serious illness. Various techniques for elimination of this bacteria were not successful as being too costly, too time-consuming, not having FDA approval or too inconvenient for consumers.

Mr. Ernest A. Voisin proposed a novel method for elimination of pathogenic organisms, such as vibriones – using hydrostatic pressure. He requested that Nicholls State University conduct a study, based on his invention, to prove or disprove efficacy of his method.

As Dr. Kilgen states, the results of the study conclusively proved that the method works, that *Vibrio Vulnificus* bacteria is eliminated (or reduced to non-detectable levels) with the use of hydrostatic high pressure method developed by the applicant. **Bacteria was eliminated at ambient temperature at a pressure of 25,000 psi with the treatment for 15 minutes.**

It is well established that the Patent Office Examiners have the burden of giving reasons with reference to supporting evidence why the specification is not enabling. It is not necessary to rule out all experimentation to meet the “how to make and use” requirement of Section 112. A reasonable amount of experimentation is allowed. In re Scarborough, 182 USPQ 298 (CCPA 1974). Consequently, if a person following the steps of the applicant, decides to apply the method of the present invention to food products derived from another water body, he/she will have this invention as a guide and

with reasonable amount of experimentation develop the process criteria applicable to that food product.

The applicant respectfully submits that his invention was successfully reduced to practice. He had studies conducted by Nicholls State University, tests done at Illinois Institute of Technology, Oregon State University and at applicant's company (see, page 3 of Dr. Kilgen's letter).

All experiments proved that the method according to the instant invention works in practice. This proof meets the requirement established by the Patent Office for a patentable invention within the meaning of 37 CFR 112. See, In re Comstock, 178 USPQ 616 (CCPA 1973); In re Naquin, 158 USPQ 317 (CCPA 1968).

In view of the above, reconsideration of rejection under 35 USC 112, first paragraph is respectfully requested.

Claims 1 – 4 and 6-7 were further rejected under 35 USC 103(a) as being unpatentable over Hirsch in view of JP 02257864 A. Examiner stated that Hirsch discloses a method of preserving scallops by exposing them to hydrostatic pressure. It is the teaching of Hirsch that treatment of scallops and shrimp at 150 MPa did not sterilize the food products.

However, as stated above, the instant invention is not directed to elimination of all living organisms in seafood, only those causing illness (pathogenic bacteria). Applicant's tests proved that at 25,000 psi and treatment for 15 minutes harmful bacteria is indeed eliminated. See Dr. Kilgen's letter.

The Examiner further contends that it would have been obvious to one of ordinary skill in the art to incorporate the teaching of Japanese reference (sterilization of bacterial

spores at 14,223 – 142,220 psi for 5-300 minutes) into the disclosure of Hirsch to arrive at the applicant's invention.

The applicant respectfully traverses this rejection and submits that a person of ordinary skill in the art is unlikely to use the teachings of Hirsch for relatively "low" levels of high pressure processing because Hirsch expressly states that the "ocean foods ... are not sterilized" at 25,000 psi. Hirsch theorizes that this is due to the fact that ocean bacteria lives at pressures of 150 MPa.

However, Hirsch offers no viable path for a person of ordinary skill in the art – according to Hirsch, low pressure does not kill the bacteria and the food product is degraded within one day of decompression. See, column 6, lines 53 – 63. Hirsch, therefore, suggests to keep the food pressurized.

The present invention does not require the food products to be kept under pressure. All that is required after pressure treatment is to keep the food products cold (similar to pasteurization). Claim 3 was amended to more specifically stress this point.

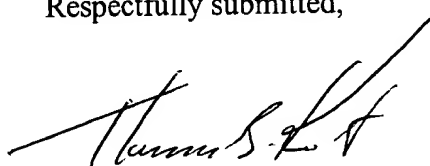
The Japanese reference also relies on sterilization for treatment of food products. It is believed that some of the criteria given in the reference (such as treatment for 300 minutes) may be too excessive for such a delicate food product as mollusk.

The applicant further amended the claims to stress the point that mollusks are treated while still in the shell, which makes the method of the present invention even more unique. No new matter was introduced, and full support to the amendments in the claims is present in the application as originally filed.

In view of the amendments and arguments presented above, the applicant respectfully submits that prima facie obviousness has not been established.

Reconsideration of the rejection and allowance of Claims 1-4 and 6-7 is respectfully solicited. Should the Examiner feel that a telephone conference would advance resolution of any issue remaining in the case, he is invited to telephone the undersigned at the phone number listed below.

Respectfully submitted,

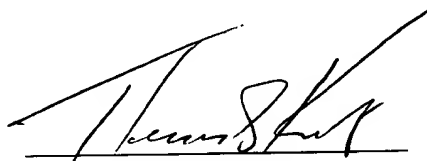


Thomas S. Keaty
Reg. No. 27,038
Keaty Professional Law Corporation
2140 World Trade Center
2 Canal Street
New Orleans, Louisiana 70130
(504) 524-2100
Attorney for Applicant

CERTIFICATE OF MAILING

I hereby certify that this response and letter from Dr. Kilgen are being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231 on the date indicated below.

10-20-99
Date


Thomas S. Keaty



Nicholls State University

October 7, 1999

To Whom It May Concern:

I would like to discuss the background, goals and objectives, and results of my research studies at Nicholls State University, which were done at the request of Ernest A. Voisin of Houma, Louisiana, to evaluate the emerging technology of hydrostatic high pressure processing (HPP) for pasteurization of live shellstock oysters. Mr. Voisin requested this work because he felt that application of high water pressure might eliminate, or reduce to non-detectable levels, a potentially pathogenic naturally-occurring marine organism, *Vibrio vulnificus*. It has long been a priority of the oyster industry, government agencies and academic researchers nationally and internationally to develop or evaluate new post harvest technologies to eliminate *V. vulnificus* from live shellstock and raw shucked oysters.

Sincerely,

Marilyn B. Kilgen, Ph.D.
Distinguished Service Professor and Head
Department of Biological Sciences
Nicholls State University
Thibodaux, LA 70310

Definitions:

Pasteurization is the application of some intervention method or treatment to **eliminate or reduce to non-detectable levels target organisms or groups of organisms** - generally pathogens or spoilage microorganisms. The product, if perishable, must still be refrigerated, and is not shelf-stable at ambient temperatures because not all of the microorganisms present are eliminated.
e.g. pasteurization of milk

Sterilization is the application of an intervention method or treatment sufficient to **kill or inactivate all living organisms or microorganisms**, and to render the product shelf stable at ambient temperatures.
e.g. sterilization of canned food products

This study produced data on the effects of HPP on *V. vulnificus* in live shellstock oysters that has never been previously obtained.

Background

Vibrio vulnificus is a naturally occurring halophilic, Gram negative, rod shaped bacterium, that ferments lactose and is found in estuarine and marine waters (13,23,29). *V. vulnificus* is considered a major component of the normal micro flora of warm ($>25^{\circ}\text{C}$) estuarine waters and thus, filter-feeding molluscan shellfish. It has been isolated from estuarine and marine waters of the U.S. gulf coast, east coast, and west coast, and on various continents (10,12,24,25,36). It has also been isolated from many seafood samples including filter-feeding molluscs, where these bacteria can adhere and multiply in the gut region, and in fish (7,8,23,28). It can be transferred from this natural environment to man directly through wound infections in estuarine waters, or by the consumption of raw or undercooked oysters by immunocompromised or "at risk" individuals (2,3,5,13,23,29). However, this naturally occurring warm water estuarine organism is the only bacteria that has been associated with any mortality in recent years, as a result of primary septicemia in "at risk" individuals through consumption of raw oysters. These deaths are very rare, and occur only in at risk individuals who are immunocompromised or have preexisting liver disease (2,3,13,23,29). These at risk individuals have only about a 1 in 25,000 chance of contracting this serious disease if they consume raw oysters contaminated with *V. vulnificus* (3); and 99% of at risk raw oyster consumers with underlying medical conditions do not acquire this disease (15). Although individuals who are considered normal healthy patients have never been reported to have contracted this disease, it has resulted in enormous negative media coverage and negative economic impact on the gulf oyster industry for the last 10 years (13, 15). Elimination of *V. vulnificus*, or decrease to non-detectable levels of this naturally occurring marine microorganism through a pasteurization process that would not impact the raw product has long been sought after because the increase in consumer confidence and marketability would result in a global economic impact on the oyster industry.

Several different techniques or strategies proposed to pasteurize or reduce the numbers of *Vibrio vulnificus* in live and processed oysters have been evaluated in the past, but each has had problems. They have included time and temperature for reduction of numbers (5), cold, freezing and heat treatments (4,5,6,15), vacuum packaging (26), use of Generally Recognized as Safe (GRAS) compounds (31), suspension relaying into offshore waters (21), ionizing irradiation (15), UV light (33), and food condiments treatment (32). Some of these strategies are somewhat effective, but may pose such problems as being too costly, too time consuming, not having FDA approval, or too inconvenient for the consumer and/or entrepreneur.

Hydrostatic high pressure processing (HPP) of foods is a novel, non-thermal means of pasteurizing food products with no or minimal heat treatments. Foods can be pre-packaged and processed under elevated water pressure up to 130,000 psi, depending on the required application. Food is placed inside a pressure vessel which contains the pressure transmitting fluid. During the process, according to Pascal's Law, the pressure has a uniform effect on everything inside the pressure vessel. Foods inside the pressure vessel in a fluid-filled container compensate for the external pressure because it is distributed in all directions equally. Therefore, HPP does not lead to large changes in volume and consequent mechanical damage to delicate food products. The two main targets for microbial inactivation include mechanical damage to the cell membrane and denaturation of one or more key enzymes.

Goal and Objectives

The goal of studies requested by Ernest A. Voisin and conducted at Nicholls State University was to pasteurize oysters to eliminate or reduce to non-detectable levels (<3 most probable number (MPN)/gram) *Vibrio vulnificus* from raw gulf coast oysters, using for the first time on live oysters, the emerging technology of cold high hydrostatic pressure processing (HPP) to avoid impacting the sensory qualities of raw oysters, and also to regain national consumer confidence in this economically important product (16,17, 18, 19).

The effects of high pressure processing (HPP) of foods, which is a non-thermal means of preserving food products with no or minimal heat treatments, has actually been known for over 90 years (9,11,27,30). However, it has just recently re-emerged as a practical technology for cold pasteurization of foods. Foods can be pre-packaged and processed under elevated water pressure up to 130,000 psi, depending on the required application. Food is placed inside a pressure vessel which contains the pressure transmitting fluid. During the process, according to Pascal's Law, the pressure has a uniform effect on everything inside the pressure vessel. Foods inside the pressure vessel in a fluid-filled container compensate for the external pressure because it is distributed in all directions equally. Therefore, HPP does not lead to large changes in volume and consequent mechanical damage to delicate food products (9,27). Oysters lend themselves very well to this processing technology because of the delicate tissue and high water content of the organism. The two main targets for microbial inactivation include mechanical damage to the cell membrane and denaturation of one or more key enzymes (9,11,27).

This technology of hydrostatic high pressure processing (HPP) will be economically important to the oyster industry because: 1) it results in exacting quality control because of instantaneous and uniform transmission of the pressure throughout the entire product, regardless of oyster volumes or sizes (9). This is not possible with either heat or ionizing irradiation; 2) it does not significantly alter sensory qualities or nutritional value due to lack of thermal processing (30); 3) it enables high productivity since the processing time does not depend on the dimensions of the product processed (isostatic); 4) it does not involve thermal damage since thermal increase is minimal ($3^{\circ}\text{C}/14,500$ psi); 5) it does not cause mechanical damage to delicate raw oysters; 6) it has minimal impact on the environment, requiring only electric energy with no waste products generated; 7) it has no negative impact on consumer acceptability (27); and finally, 8) consumer confidence in oysters could be restored for greatly increased market value (18, 19).

Results

The first preliminary or pilot tests were run at the Illinois Institute for Technology's (IIT) Center for Food Safety and Technology (CFST) in collaboration with Ernest A. Voisin. Tests on a larger scale were later run at Oregon State University (17). Most recent tests were run at Motivati Seafoods, Inc. in Houma, LA in their commercial level hydrostatic high pressure processor, developed in conjunction by them and Flow International, Inc. (16). These studies on live shellstock oysters using standard FDA approved methods (1, 20, 22, 30), on approximately 60 oysters per sample run showed that hydrostatic high pressures in the commercial machine reduced the naturally occurring or ambient levels of *Vibrio vulnificus* in the live shellstock oysters to "non-detectable levels" at a pressure of 25,000 psi for 15 minutes.

Analysis of the samples were done at days 0, 7, 14 and 21 post high pressure processing to confirm that *V. vulnificus* does not repair from the HPP damage during 3 weeks cold storage at approximately 35-40°F in commercial cold storage. The control or untreated oysters had ambient or naturally occurring levels of 460,000 most probable number (MPN) *Vibrio vulnificus*/gram (16).

***Note:** The United States Food and Drug Administration (USFDA) in conjunction with the Interstate Shellfish Sanitation Conference (ISSC) have defined "non-detectable levels" of *V. vulnificus* to be <3 Most Probable Number (MPN)/gram. (Model Ordinance, 1997, Chap. XI, J (i) (i), p. 89. (See attached pages from ISSC/FDA Model Ordinance.)

In addition, it was observed by Kilgen et al., 1999 (17) during the pilot tests requested by Ernest A. Voisin at the Illinois Institute of Technology's (IIT's) Center for Food Safety and Technology (CFST), that the oyster adductor muscle connective tissue attachment at the shell denatured to gel formation at pressures as low as 20,000 psi for 15 minutes. The denaturation of other types of muscle proteins, including actin and myosin, and connective tissues to a gelatin transition have been documented in the past (9, 27), and are a result of disruption of non-covalent interactions in tertiary protein structures. **The potential commercial utilization of this chemical denaturation for mechanical shucking of live shellstock oysters has not been previously tested or observed.** This has great commercial and economic implications since approximately 80% of the cost of a shucked oyster is in the labor intensive hand shucking process, and oyster damage due to shucking causes water loss or "bleeding" (35).

Overall, the novel use of hydrostatic high pressure processing for the pasteurization of live shellstock oysters to eliminate or reduce to non-detectable levels, the naturally-occurring marine organism *Vibrio vulnificus*, and other vibrio species, and for mechanical shucking of shellstock oysters, will result in significant benefit to the global economy of the oyster and other molluscan shellfish industries (16, 17, 35).

Literature Cited:

1. American Public Health Assoc. 1985. Recommended procedures for the examination of seawater and shellfish. 5th Ed. A.E. Greenberg, D.A. Hunt, Eds. APHA, Wash, D.C. p.144.
2. CDC. 1996. *Vibrio vulnificus* Infections Associated with Eating Raw Oysters. MMWR 45(29):621-624.
3. CDC. 1993. *Vibrio vulnificus* Infections Associated with Raw Oyster Consumption - Florida, 1981-1992. MMWR. 42:405-407.
4. Cook, D.W. and A.E. Ruple. 1992. Cold Storage and mild Heat Treatment as Processing Aids to Reduce the Numbers of *Vibrio vulnificus* in Raw Oysters. J Food Prot. 55(12)985-989.
5. Cook, D.W. 1994. Effect of Time and Temperature on Multiplication of *Vibrio vulnificus* in Postharvest Gulf Coast Shell stock oysters. Appl Environ Microbiol. 60(9) 3483-3484.
6. Cook, D.W. 1997. Refrigeration of Oyster Shell stock: Conditions Which Minimize the Outgrowth of *Vibrio vulnificus*. J. Food Prot. 60(4) 349-352.
7. DePaola, Angelo, G.M. Capers and D. Alexander. 1994. Densities of *Vibrio vulnificus* in the Intestines of Fish from the U.S. Gulf Coast. Appl Environ Micro 60(3):984-988.

8. Groubert, T.N. and J.D. Oliver. 1994. Interaction of *Vibrio vulnificus* and the Eastern Oyster, *Crassostrea virginica*. Journal of Food Protection. 57(3):224-228.
9. Farr, D. High pressure technology in the food industry, a review. Trends in Food Science and Technology, July 1990, pp14-16.
10. Hoi, L., J.L. Larsen, I. Dalsgaard, and A. Dalsgaard. 1998. Occurrence of *Vibrio vulnificus* biotypes in Danish Marine Environments. Appl. Environ. Microbiol. 64:7-13.
11. Hoover, D.G., C. Metrick, A. Papineau, D. Farkas, and D. Knorr. March, 1989. Biological effects of high hydrostatic pressure on food microorganisms. FoodTechnology: 99-107
12. Kaysner, Charles, C. Abeyta, Jr., M.M. Wekell, A. DEPaola, R.F. Stott, and J.M. Leitch. 1987. Virulent strains of *Vibrio vulnificus* isolated from estuaries of the United States West Coast. Appl. Environ. Microbiol. 53:1349-1351.
13. Kilgen, M.B. 1991. Public health issues stemming from water-borne pathogens in the Barataria-Terrebonne estuary. Barataria-Terrebonne National Estuary Program - Scientific-Technical Committee Data Inventory Workshop Proceedings pp 202-219.
14. Kilgen, M.B. 1993. Cost-benefit aspects of irradiation processing for Louisiana oysters. In: Proceedings of an Internat. Symp. on Cost-Benefit Aspects of Food Irradiation Processing, pp 89-101. IAEA, Vienna.
15. Kilgen, M.B. and M.T. Hemard. 1996. Evaluation of commercial irradiation and other processing methods for *Vibrio vulnificus* control in Louisiana oysters. Proceedings of the 19th and 20th Annual Conferences of the Tropical and Subtropical Seafood Science and Technology Society of the Americas. pp. 300-310.
16. Paris, C. L., A. Rhodes, and M. B. Kilgen. 1999. Evaluation of commercial hydrostatic high pressure processing for mechanical shucking and *Vibrio vulnificus* control in Louisiana shellstock oysters. Abstract to the American Society for Microbiology, South Central Branch. October, 1999, New Orleans, LA
17. Kilgen, M.B., J. Bourgeois and C. Porche. 1999. Hydrostatic high pressure processing of Louisiana oysters for *Vibrio vulnificus* control and mechanical shucking. Abstract to the Louisiana Academy of Sciences, February, 1999, Monroe, LA
18. Levy, A.S. and S. Fein. 1995. Consumer perceptions of food safety problems and reported practices. Division of Market Studies, Center for Food Safety and Applied Nutrition, FDA.
19. Lin, J., W. Milon, E. Babb and R. Degner. 1991. Consumer perceptions of shellfish related safety risks: Results from east coast focus groups. Food and Resource Economics Department, University of Florida.
20. Massad, George and J.D. Oliver. 1987. New Selective and Differential Medium for *Vibrio cholerae* and *Vibrio vulnificus*. Appl Environ Micro. 53(9):2262-2264.
21. Motes, M,L, and A.DePoala. 1996. Offshore Suspension Relaying to Reduce Levels of *Vibrio vulnificus* in Oysters (*Crassostrea virginica*). Appl Environ Micro 62(10):3875-3877.
22. Oliver, J.D., K. Guthrie, J. Preyer, A. Wright, L.Simpson, R. Seibling, J.G. Morris, Jr. 1992. Use of Colistin-Polymyxin B-Cellobiose Agar for Isolation of *Vibrio vulnificus* from the Environment. Appl Environ Micro. 58(2): 737-739.
23. Oliver, J.D. 1989. *Vibrio vulnificus*. 569-599. In M. Doyle (ed.), Food-borne Bacterial Pathogens. Marcel Dekker, Inc., New York.

24. Oliver, J.D., R.A. Warner, and D.R. Cleland. 1982. Distribution and ecology of *Vibrio vulnificus* and other lactose-fermenting marine vibrios in coastal waters of the southeastern United States. Appl. Environ. Microbiol. 44: 1404-1414.
25. Oneill, K.R., S.H. Jones, and D.J. Grimes. 1992. Seasonal incidence of *Vibrio vulnificus* in the Great Bay Estuary of New Hampshire and Maine. Appl. Environ. Microbiol. 58: 3257-3262.
26. Parker, R.W., E.M. Maurer, A.B. Childers, and D.H. Lewis. 1994. Effect of Frozen Storage and Vacuum-Packaging on Survival of *Vibrio vulnificus* in Gulf Coast Oysters (*Crassostrea virginica*). J Food Prot. 57(7): 604-606.
27. Rovere, P. 1995. The third dimension of food technology. Tecnologie Alimentari 4: 1-6.
28. Ruple, A.D. and D.W. Cook. 1992. *Vibrio vulnificus* and Indicator Bacteria in Shellstock and Commercially Processed Oysters from the Gulf-Coast. J Food Prot. 55(9): 667-671.
29. Seafood Safety. 1991. National Academy Press, Washington, D.C., p. 292-293.
30. Styles, M.F., D.G. Hoover and D.F. Farkas. 1991. Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. J. of Food Science. 56(5): 1404-1407.
31. Sun, Yi and J.D. Oliver. 1994. Effects of GRAS Compounds on Natural *Vibrio vulnificus* Populations in Oysters. J Food Prot. 57(10): 921-923.
32. Sun, Yi and J.D. Oliver. 1995. Hot Sauce: No Elimination of *Vibrio vulnificus* in Oysters. J Food Prot. 58(4) 441-442.
33. Tamplin, M.L. and G.M. Capers. 1991. Persistence of *Vibrio vulnificus* in Tissues of Gulf Coast Oysters, *Crassostrea virginica*, Exposed to seawater Disinfected with UV Light. Appl Environ Micro. 58: 1506-1510.
34. Tamplin, M.L., A.L. Martin, A.D. Ruple, D.W. Cook and C.W. Kaspar. 1991. Enzyme Immunoassay for Identification of *Vibrio vulnificus* in Seawater, Sediment, and Oysters. Appl Environ Micro. 57(4): 1235-1240.
35. Voisin, M. 1998. Personal communication.
36. Wright, Anita, R.T. Hill, J.A. Johnson, M. Roghmen, R.R. Colwell and J.G. Morris. 1986. Distribution of *Vibrio vulnificus* in the Chesapeake Bay. Appl. Environ. Microbiol. 62: 717-724.